TEST FOR NITRATE NITROGEN

IN TOMATO PLANTS

FREEMAN S. HOWLETT

DALE W. KRETCHMAN

DEPARTMENT OF HORTICULTURE

The Ohio Agricultural Research and Development Center
Wooster, Ohio

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The presence of nitrate-nitrogen in plant tissue has for many years been indicated by utilizing a chemical solution containing diphenylamine. Although the test is not quantitative it has distinct value in indicating the presence of nitrate at a fairly wide range of concentration. In view of this fact it has served a useful purpose in tomato culture by indicating the need for side dressings with nitrogen during the course of plant growth particularly in the case of the greenhouse tomato.

Principle involved in the test

Nitrate nitrogen reacts with diphenylamine to develop a blue color. The intensity of the blue is related to the quantity of nitrate nitrogen present particularly when nitrate is present at low concentrations. However, at high concentration, a deep blue intensity results which apparently represents a wide range of nitrate nitrogen.

In order to obtain a satisfactory test the cell wall tissue must be disrupted and the cell contents exuded in order that the nitrate contained in the cells can react with the diphenylamine. The sulfuric acid in which the diphenylamine is dissolved serves as the agent to break down the cell walls when the solution and plant sections are stirred. It is important that a sufficient quantity of the reacting solution be applied in order to react with the nitrate present. Too much plant tissue and too little reacting solution may prevent a satisfactory test. Furthermore sufficient time must be given for the breakdown of the cell tissue. Two or more minutes are required. Time must be given for the maximum intensity of blue color to develop. Shortly after this color has developed the color changes to a greenish blue indicating that the reaction has been completed. In view of these facts care must be taken in carrying out the test in accordance with the directions given below.

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Directions for the Test

1. Select a very young leaf at the top of the plant. This leaf should be no longer than 6 inches and preferably 3 to 4 inches in length. Four thin whole cross sections of the petiole (stem) of this leaf are removed with a safety razor blade. The blade must have been washed free of nitrate nitrogen following its previous use as nitrate will remain dried on its surface for some time.

These sections are placed in a dry and thoroughly clean well of the spot plate. Again the well of this spot plate must be free from nitrate present from the previous test of from any contamination from hand.

A sufficient amount of the diphenylamine solution is then added by means of a pipette. Four medium to large drops should be applied depending upon the diameter and thickness of the sections. No more than 6 drops should be necessary for thick sections. It should be kept in mind that only the petioles of very young soft leaves are to be utilized.

The mixture is stirred until the maximum blue color is observed. The changes occ rring should be watched carefully as a small amount will show blue for only a very short time before the color changes to a dirty green-blue.

The length of time required to turn maximum blue will depend on several factors as the (1) amount of nitrate present (2) temperature of the solution (3) age of the tissue being tested (4. and age of the test solution.

In all tests at a given time a similar number of drops of the solution and sections of the same thickness showed be utilized.

The relative amounts of nitrate nitrogen are to be indicated by the following symbols:

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0 - None present
1 - Recognizable trace
2 - Small amount - very light blue
3 - Moderate amount, solution will be darker but white background of plate will still be visible - moderate blue
4 - Considerable amount - white background of spot plate not visible - solution deep blue.
5 - Large amount - solution blue-black

As previously stated a rather wide range of nitrate is indicated by reactions giving no. 4 and no. 5 intensities.

Composition of the Diphenylamine Solution

1 gram of diphenylamine is dissolved in 100 milliliters of 75% sulfuric acid. Concentrated chemically pure sulfuric acid should be utilized.

The solution should be stored in a green or amber dropping bottle or in the dark when not in use. The solution should be tested for activity before use after its color has appreciably changed.

If kept in the refrigerator when not in use the reagent should be allowed to warm somewhat before utilizing it to test for nitrate nitrogen.

Certa n additional statements should be made relative to the use of this test as follows:

Application of Test to Tomato Culture

It should be kept in mind that the test is only for nitrate nitrogen. It is of no value for testing for ammonium nitrogen. If ammonium nitrate has been applied the solution can react only with the nitrate fraction within the plant. This fact means that if ammonium nitrate has been used as the source of nitrogen more soluble nitrogen available for plant growth exists within the plant tissue than is indicated by the nitrate test. If ammonium sulfate is utilized as the source of nitrogen the use of diphenylamine is of little value.

Frequency of the test

The test should be made at least once weekly from the same plant or plants in very close proximity to each other. Thus tests should be made

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in several locations within a house and the results averaged to provide an indication of the general nitrate status.

Time of day

Although the test may be made at any time of day it is suggested that it be carried out during the afternoon, possibly around 3 p.m. Theoretically at this time the nitrate content should be lowest.

Cleanliness of operation involved in testing

Obviously provisions should be taken to prevent presence of nitrate on the surface of the leaf petiole being tested. The leaf should not be laid on a bench following removal from the plant and one's hands must be free of nitrate nitrogen prior to cutting the sections and while making the test - (except what may come out of the cells of the section during cutting).

Interpretation of the Results of the Test

The presence of nitrate in the petioles of the uppermost leaves indicates that a continuing supply of nitrate is entering the plant. Such nitrate in this particular location indicates that a supply is available for growth at this point. The test indicates that a source of nitrogen is available which upon changing to the NH_2 fraction, reacts chemically with reserve carbohydrates to form amino acids and protein.

Lack of any nitrate at this location (in case nitrate has been applied as the source of nitrogen to the soil) obviously means that growth restriction will shortly place. Nitrogen from the breakdown of proteins in the lower basal leaves will in part supplement the nitrate added to the soil but usually an insufficient supply from this breakdown for satisfactory growth is available. For this reason nitrogen should be added to the soil as a side dressing in order to make certain that nitrate nitrogen reaches the uppermost leaves very promptly.

From past experience it seems advisable to make no additions of

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nitrogen to the soil while the petioles of the tip leaves show a very blue to a blue-black condition. It should be remembered that the nitrogen available for growth at the tip of the plant results from that applied in the ammonium form as well as the nitrogen which has been broken down from the proteins in the lower more basal leaves. The soluble nitrogen which becomes available from proteins is translocated to the tip of the plant. Too much emphasis cannot be given to the fact that the very blue to the blue-black condition represents a nitrate excess over and above current requirements for nitrogen.

On the basis of present evidence it appears that sufficient nitrate nitrogen is present if continously a condition not to exceed no. 3 is present. If the test one day is equivalent to no. 1 and the next day to 0 or the reverse, a side dressing of a nitrogen carrying fertilizer should be made very promptly.

It should be pointed out that the primary value of the diphenylamine test for nitrate nitrogen is for indicating the desirability of adding a side-dressing of nitrogen to the plants. For ascertaining the nutritional status of the plants and the amount of both nitrate nitrogen, ammonium nitrogen, soluble organic and protein nitrogen, leaf analysis which includes the total amount of all nitrogen is necessary.

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